

Toxicity & Teratogenicity Studies in Avian Embryos
Glycine No Date

FDA Contract #72-345

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GLYCINE

TOXICITY and TERATOGENICITY STUDIES
in Avian Embryos

FDA Contract #72-345

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GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (~~post~~^{RE}-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
 2. Linear regression analyses + chi square test of linearity.
 - a. % response against dose
 - b. % response against log dose
 - c. log % response against dose
 - d. arcsin transformation against dose
 - e. arcsin transformation against log dose
 3. Log dose against Probit using Finney's maximum likelihood method.
 - a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
 4. One-way analyses of variance.
 5. Linear regression with replication.
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Glycine (71-42) was solublized in water for use in the test protocols. The maximum dose levels of 200 mg/kg was obtained with a solution of 200 mg/ml.

MORTALITY

The mortality data obtained in the four test procedures are shown in Tables 1 - 4. Chi-square analyses of these data indicated that the injection of glycine into the air cell at either 0 or 96 hrs produced a significant increase in mortality of chicken embryos at dose levels of 80 and 200 mg/kg, respectively (Table 5). Yolk administration did not significantly increase embryo mortality at any of the dose levels employed.

Probit analyses of the mortality data obtained in the four test protocols failed to yield a statistical linear relationship between log dose and probit of mortality (Table 6). These data suggest that relatively high concentrations of glycine are required to produce a significant increase in mortality rate of chicken embryos.

TERATOLOGY

The incidence of abnormal embryos and the occurrence of H-S-V-L abnormalities are shown in Tables 1 - 4. Glycine was not-teratogenic in these studies. Chi-square analyses of the data obtained on the occurrence of abnormalities failed to indicate a statistically significant difference between the water injected controls and those receiving glycine at levels up to 200 mg/kg (Table 7). Probit analyses of the incidence of abnormalities were likewise not significant (Table 8). The occurrence of H-S-V-L abnormalities with the injection of glycine was not found to be significantly different by chi-square analyses from that obtained in the water injected controls (Table 9). The individual teratology findings obtained in the four test protocols are shown in Table 10.

STUDIES on the TOXICITY and TERATOGENICITY
of GLYCINE

SUMMARY and CONCLUSIONS

The administration of glycine (71-42) at levels of 80 and 200 mg/kg significantly increased embryo mortality ($P < 0.05$) when aircell administration was employed at 0 and 96 hrs, respectively. Yolk injections of glycine were not embryo toxic at either of the injection times at levels up to 200 mg/kg.

Glycine was not found to be teratogenic in these studies. Statistical evaluations of the data obtained in the 4 test protocols did not indicate a significant increase in the occurrence of abnormal embryos with the administration of glycine.

TABLE 1
GLYCINE in WATER
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category							
						Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functional % #	
				Total % #	H-S-V-L % #								
200.0	215	15.81	34	1.86 4	0.46 1			0.46 1		1.39 3			
80.0	177	25.98	46	2.25 4	1.12 2			1.12 2		1.12 2	1.12 2		
40.0	175	22.28	39	1.14 2	1.14 2			1.14 2					
8.0	174	22.98	40	2.29 4	0.57 1			0.57 1		1.14 2	0.57 1		
4.0	176	19.31	34	0.00 0	0.00 0								
0.0	156	15.38	24	0.64 1	0.64 1	0.64 1				0.64 1			
drilled	120	15.00	18	0.00 0	0.00 0								
untreated	445	10.33	46	0.44 2	0.00 0					0.22 1	0.22 1	0.22 1	

SUMMARY - ALL DOSE LEVELS

917	21.05	193	1.53	14	0.65	6			0.65	6		0.76	7	0.33	3	
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TABLE 3
GLYCINE in WATER
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category													
						Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functional % #							
				Total % #	H-S-V-L % #														
200.0	98	38.77	38	3.06	3	2.04	2			2.04	2			1.02	1	1.02	1		
80.0	59	52.24	31	0.00	0	0.00	0												
40.0	60	41.66	25	1.66	1	3.33	2	1.66	1		1.66	1							
8.0	60	46.66	28	3.33	2	0.00	0							3.33	2				
4.0	60	26.66	16	0.00	0	0.00	0												
0.0	97	45.36	44	2.06	2	1.03	1	1.03	1					1.03	1				
pierced	116	23.27	27	4.31	5	2.58	3			1.72	2	0.86	1	0.86	1	1.72	2		
untreated	445	10.33	46	0.44	2	0.00	0							0.22	1	0.22	1	0.22	1

SUMMARY - ALL DOSE LEVELS

337	40.95	138	1.78 6	1.19 4	0.30 1		0.89 3		0.89 3	0.30 1	
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TABLE 4
GLYCINE in WATER
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category						
						Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functional % #
				Total % #	H-S-V-L % #							
200.0	162	25.92	42	1.85 3	1.23 2	1.23 2					0.61 1	
80.0	125	23.20	29	0.00 0	0.00 0							
40.0	124	26.61	33	0.80 1	0.80 1	0.80 1						
8.0	123	30.08	37	0.81 1	0.81 1			0.81 1				
4.0	124	33.87	42	0.80 1	0.80 1			0.80 1				
0.0	156	24.35	38	0.00 0	0.00 0							
pierced	196	22.95	45	1.02 2	1.53 3	0.51 1		0.51 1	0.51 1	0.51 1		0.51
untreated	445	10.33	46	0.44 2	0.00 0					0.22 1	0.22 1	0.22 1

SUMMARY - ALL DOSE LEVELS

658	27.81	183	0.91 6	0.76 5	0.46 3		0.30 2			0.15 1	
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TABLE 2
GLYCINE in WATER
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category						
						Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functiona % #
				Total % #	H-S-V-L % #							
200.0	154	22.72	35	1.29 2	1.29 2	0.64 1		0.64 1				
80.0	117	12.82	15	0.00 0	0.00 0							
40.0	118	10.16	12	0.00 0	0.00 0							
8.0	120	16.66	20	0.83 1	0.00 0						0.83 1	
4.0	118	13.55	16	0.00 0	0.00 0							
0.0	153	11.11	17	0.65 1	0.65 1	0.65 1						
drilled	121	11.57	14	0.00 0	0.00 0							
untreated	445	10.33	46	0.44 2	0.00 0					0.22 1	0.22 1	0.22 1

SUMMARY - ALL DOSE LEVELS

627	15.63	98	0.48 3	0.32 2	0.16 1		0.16 1			0.16 1	
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TABLE 5
GLYCINE
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
4.000	0.636	0.180	4.724*(less)	2.615
8.000	2.575	1.329	0.000	0.873
40.000	2.121	0.003	0.083	0.085
80.000	4.996*	0.058	0.498	0.007
200.000	0.001	6.558*	0.618	0.037
All Doses (DF)	9.877 (5)	12.208*(5)	9.893(5)	5.108(5)

* Probability < 0.05 - 0.005.

TABLE 6
 GLYCINE
 PROBIT ANALYSES - MORTALITY

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

TABLE 7
GLYCINE
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
4.000	0.004	0.017	0.150	0.013
8.000	0.608	0.294	0.001	0.014
40.000	0.010	0.017	0.180	0.013
80.000	0.579	0.018	0.142	0.000
200.000	0.302	0.000	0.000	1.271
All Doses (DF)	5.538(5)	3.985(5)	3.810(5)	4.864(5)

TABLE 8
GLYCINE
PROBIT ANALYSES - ABNORMALITIES

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

TABLE 9
GLYCINE
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
4.000	0.004	0.017	0.059	0.013
8.000	0.400	0.015	0.059	0.014
40.000	0.010	0.017	0.150	0.013
80.000	0.012	0.018	0.063	0.000
200.000	0.240	0.000	0.000	0.466
All Doses (DF)	2.561 (5)	5.474 (5)	3.387 (5)	2.987 (5)

GLYCINE in WATER TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO. D E S C R I P T I O N
Untreated Control		445	2	1 hypopigmentation-down
				1 cachexia; aplasia-down
Pierced Control - 0 hrs		116	5	1 dwarfism; hypopigmentation-down
				1 hypopigmentation-down
				1 fusion failure-abdomen
				1 abnormal curvature-hindlimb
				1 celosomia-abdomen
Pierced Control - 96 hrs		196	2	1 anophthalmia ; dysgnathia-beak; abnormal curvature- hindlimb
				1 cachexia; aplasia-down; fusion failure-abdomen
Air Cell - 0 hrs	200.0 mg/kg	215	4	1 fusion failure-abdomen
				1 dwarfism
				2 agenesis-down
	80.0	177	4	2 dwarfism; hypopigmentation-down
				1 fusion failure-abdomen
				1 celosomia-abdomen
	40.0	175	2	2 fusion failure-abdomen

TABLE 10
GLYCINE in WATER
TERATOGENIC FINDINGS

TERATOGENIC FINDINGS													
TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS									
				NO.	D	E	S	C	R	I	P	T	I
Air Cell - 0 hrs	8.0 mg/kg	174	4	1	agenesis-down; dwarfism								
				1	agenesis-down								
				1	hemorrhage								
				1	fusion failure-abdomen								
	0.0	156	1	1	anophthalmia; acrania; abnormal shortening-maxilla; dwarfism								
Air Cell - 96 hrs	200.0	154	2	1	fusion failure-abdomen								
				1	dysgnathia-beak								
	8.0	120	1	1	hemorrhage								
	0.0	153	1	1	anophthalmia; dysgnathia-beak								
Yolk - 0 hrs	200.0	98	3	1	dwarfism; celosomia-abdomen								
				1	celosomia-abdomen								
				1	umbilical cord around fetus								
	40.0	60	1	1	anophthalmia; dysgnathia-beak; celosomia-abdomen								
	8.0	60	2	1	agenesis-down								
				1	dwarfism								
	0.0	97	2	1	agenesis-down								
				1	exencephaly; dysgnathia-beak								

TABLE 10

GLYCINE in WATER TERATOGENIC FINDINGS

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